

Prevalence of Secondary Amyloidosis in Asian North Indian Patients with Rheumatoid Arthritis

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ABSTRACT. Objective. To study the prevalence of secondary amyloidosis in Asian North Indian patients with rheumatoid arthritis (RA) and to determine its clinical significance.

Methods. RA patients with disease duration > 5 years were included in this prospective study over a 2 year period. Abdominal subcutaneous fat pad aspiration (ASFA) was performed, and smears were stained with Congo red and observed for apple-green birefringence under polarized light microscopy. The amyloid deposits were graded from 1+ to 3+. Clinical, radiological, and laboratory variables of the patients were correlated with the presence or absence of amyloidosis.

Results. Thirty out of 113 patients were positive for amyloid by ASFA (26.5%). Out of these, 8 patients had features suggestive of clinical amyloidosis in the form of proteinuria, organomegaly, or symptomatic gastrointestinal involvement. In another 22 patients amyloidosis was subclinical. The majority of patients with clinical amyloidosis had either 2+ or 3+ deposits.

Conclusion. Abdominal fat amyloid deposits are not uncommon in adult Asian North Indian patients with RA. However, only one-fourth of patients had evidence of clinical amyloidosis. A longer followup and a larger multicentric collaborative study is needed to determine the significance of subclinical amyloid deposits. (J Rheumatol 2003;30:948-51)

Key Indexing Terms:

INFLAMMATORY ARTHRITIS

COMPLICATIONS

AMYLOIDOSIS

Reactive amyloidosis is an important longterm complication of rheumatoid arthritis (RA). The development of clinical amyloidosis, as evidenced by renal or gastrointestinal involvement, is associated with a poor prognosis and causes death in 2-9% of patients¹⁻⁴. There is a growing impression that the incidence of amyloid secondary to RA is declining, arguably due to better control of inflammation⁵⁻⁶.

The prevalence of secondary amyloidosis in RA in the Western literature varies between 5 to 78%, depending on patient variables, ethnic group, method used for detection, and whether the study was prospective or retrospective⁷⁻¹⁰. Abdominal subcutaneous fat pad aspiration (ASFA) is a sensitive, minimally invasive, outpatient based, quick, easy, reproducible and repeatable procedure for the detection of amyloid¹¹⁻¹⁶.

In India, rheumatology services are scarce, resulting in considerable delay in institution of appropriate drug treatment of RA. Therefore, the prevalence of complications of disease including amyloidosis is likely to be high. As there are no data available from our country, we studied the preva-

lence of secondary amyloidosis in Asian North Indian patients with RA by ASFA and determined its clinical significance.

MATERIALS AND METHODS

Patients. Patients with RA seen in our clinical immunology clinic (Lucknow, India), and fulfilling American College of Rheumatology criteria¹⁷, with disease duration of more than 5 years were included in this prospective study. Patients with onset of disease before 16 years of age and those with a co-existing chronic disorder that by itself could lead to secondary amyloidosis, e.g., tuberculosis, were excluded from the study.

Abdominal subcutaneous fat pad aspiration (ASFA). Abdominal fat tissue was aspirated with a 16-gauge needle connected to a 10 ml syringe. Fat smears were made on glass slides and stained with Congo red. The slides were observed under polarized light for apple-green birefringence, which is characteristic of amyloid. The smears were examined and scored independently by 2 experienced pathologists. The procedure was standardized using aspirates from patients known to have amyloidosis (histopathologically) and healthy individuals. The amount of amyloid deposit was scored visually as: Negative = no detectable amyloid, or very small and isolated Congo red positive deposits; (+) = linear amyloid deposits in < 25% of tissue fragments; (++) = linear deposits in > 25% of fragments or massive amyloid deposit in < 25% of fragments; (+++) = linear deposits in > 75% of fragments or massive amyloid deposition in > 25% of tissue fragments⁷. In some patients who had ++ or +++ deposit immunofixation was done to exclude AL amyloid.

Patient variables. Age, sex, age at onset of disease, duration of disease, use of nonsteroidal antiinflammatory drugs/disease modifying antirheumatic drugs (DMARD)/corticosteroids, presence of anemia, pedal edema, organomegaly, functional status (Steinbrocker), number of swollen and tender joints, and presence of extraarticular features and joint deformities were recorded. Laboratory variables included hemoglobin, erythrocyte sedimentation rate by Westergren method, C-reactive protein (turbidimetry;

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Behring, Germany), rheumatoid factor (latex agglutination; Ranbaxy, India), serum creatinine, spot urine and 24 h urine protein (where indicated) excretion and presence of erosions on radiographs. Rectal and kidney biopsy and tests for malabsorption were done where indicated.

RA patients with proteinuria or malabsorption or previous documentation of amyloidosis by histopathology and having a positive ASFA test were classified as having "clinical amyloidosis." The rest were categorized as having "subclinical amyloidosis."

Statistical analysis. Chi-square test and unpaired student's t test were done for intergroup comparisons.

RESULTS

Amyloid deposits were detected in 30 out of 113 (26.5%) patients. Twenty-three, 5, and 2 patients had 1+, 2+ and 3+ deposits, respectively. Both patients with 3+ and 2 patients with 2+ amyloid deposits had clinical amyloidosis. Three of the 7 patients with 3+ or 2+ amyloid did not show any band on serum immunofixation.

Of the patients who tested positive for amyloid by ASFA, 8 patients had clinical amyloidosis. Seven patients had nephrotic range proteinuria and one patient had intestinal involvement in the form of malabsorption. None of the patients with nephrotic range proteinuria were taking parenteral gold therapy. Kidney biopsy was done in 3 patients, all of whom showed amyloidosis of kidney. None of the patients had renal insufficiency. Hepatosplenomegaly was present in 6 cases. Duodenal biopsy in the patient with malabsorption was positive for amyloidosis. Of those who were amyloid negative, 4 patients had proteinuria < 500 mg/day and one of these patients was taking parenteral gold. There was no significant difference in the clinical, laboratory or radiological variables of RA between the patients who had clinical versus those who had subclinical amyloidosis.

Table 1 shows the comparison of clinical and laboratory variables between the amyloid positive and negative group. DMARD use was higher among patients who were positive for amyloid ($p < 0.05$). More patients who were positive for amyloid had functional disability of Grade III/IV as compared to the group who were negative for amyloid; however, the difference was not statistically significant. There was no significant difference in the other variables including presence of erosions and duration of disease.

Seven patients with disease duration less than 6 years were detected to be positive for amyloid. One patient with disease duration of 4 years and aggressive disease had unexplained proteinuria that was positive for amyloid.

DISCUSSION

Amyloid deposits were present in about one-fourth of patients with RA after a mean disease duration of 10 years. Of these, only 25% had clinical amyloidosis.

Amyloidosis was detected in 15–20% of patients of RA at autopsy^{3,4,6}. A low prevalence of about 8%¹⁸ to as high as 78% using tru-cut abdominal fat pad biopsy has been

Table 1. Comparison of clinical and laboratory variables of amyloid positive and negative patients.

	Amyloid Positive	Amyloid Negative
Total patients, no. (%)	30/113 (26.5)	83/113 (73.5)
Age, yrs, mean \pm SD	46 \pm 8.7	45 \pm 10.9
Male:female	8:22	16:67
Age at disease onset, yrs, mean \pm SD	36 \pm 10	35 \pm 9.9
Disease duration, yrs, mean \pm SD (range)	10 \pm 5 (4–22)	10 \pm 4.5 (5–25)
Mean SJC \pm SD	5 \pm 5	4 \pm 5.4
Mean TJC \pm SD	8 \pm 8	6 \pm 6.5
Deforming disease	15 (50)	49 (59)
Steinbrocker I II III IV	6 14 8 2	21 48 10 4*
Erosive disease, no. (%)	25 (83.3)	58 (69.8)
Proteinuria, no. (%)	7 (23.3)	4 (4.8)***
Receiving DMARD, no. (%)	25 (83.3)	51 (61.4)**
Receiving steroids, no. (%)	18 (60)	46 (55.4)
RF positivity, no. (%)	25 (83.3)	64 (77)
CRP, mean \pm SD	4.8 \pm 4.0	3 \pm 3.1

* $p = 0.05$, ** $p < 0.05$, *** $p \leq 0.01$ (Fisher's exact test). RF: rheumatoid factor; CRP: C-reactive protein, mg/dl; DMARD: disease modifying antirheumatic drugs; SJC: swollen joint count; TJC: tender joint count; NS: not significant; SD: standard deviation.

reported¹⁰. The study by Kobayashi, *et al*⁹ demonstrated the presence of amyloid in 13.3% of gastroduodenal mucosa samples in a group of 407 Japanese patients with RA. Our prevalence data are comparable with the study by Casanovas, *et al*, which shows a prevalence of 19%⁷, but more than 7% as reported by Mansoury, *et al*⁸. All 3 studies included patients with disease duration greater than 5 years, used subcutaneous fat pad aspiration, and thus are comparable. The difference in the prevalence of amyloid may reflect the change in the paradigm that has occurred in the therapeutic approach to RA over the last 2 decades. The former study⁷ recruited patients during the early 1980s and would have likely followed the "go slow, go low" pyramidal algorithm of therapy of RA, whereas the patients in the latter study⁸ were recruited in the late 1990s and would have received earlier and more aggressive therapy.

These observations suggest that improvement in management of RA and adequate control of disease activity lead to a decrease in the incidence of amyloid. Many of our patients received DMARD late in the course of their disease (5–6 yrs), primarily due to delayed referral. Further, patient compliance is poor due to economic constraints. Also, the mean duration of disease in patients with amyloidosis is only 10 years in our study as compared to nearly 17 years in the other 2 studies, likely reflecting uncontrolled disease activity.

It is well known that clinically manifest amyloidosis is associated with a poor prognosis and a lower survival rate, especially with renal involvement^{1–4}. Seventy-five percent

of patients with amyloidosis in the present study had subclinical amyloidosis. Other studies^{7-9,18} have also reported similar observations.

What is the relevance of subclinical detection of amyloidosis in RA? In a recent longitudinal study, only 10% of patients went on to develop clinical amyloidosis after a mean followup of 10 years. The latency period between a positive ASFA test result and the first clinical manifestation of systemic amyloidosis was 62 months (range 18–164)⁷. However, even small and minute deposits of amyloid in patients have been associated with a higher mortality⁹. The data for amyloid deposits in ankylosing spondylitis are similar. It was observed that the prevalence of amyloid was lower (7%) and that the deposition remained subclinical in the majority of patients after many years of followup¹⁹. Thus, at present, there are no data to support therapy for amyloidosis in these patients.

We chose 5 years disease duration as the cutoff as amyloidosis is known to be a late complication of RA, occurring after 10–15 years of disease. Two recent studies have also used the same inclusion criteria (Table 2). However, it has been rarely reported as early as 2–3 years after active RA^{20,21}. We also had one patient with disease duration of 4 years who had unexplained proteinuria and was detected to be amyloid positive. In our study, a majority of patients with clinical amyloid had a higher amount of

deposits. It has been postulated that patients with more extensive fat deposits of amyloid have a greater risk of developing clinical amyloidosis⁷. However, ASFA is at best a semiquantitative method for the characterization of amyloid. Are quantitative methods for detection of amyloid better?

Quantification of amyloid A protein in abdominal fat aspirate by ELISA has been described²² but could not be done in our study. However, quantification of amyloid and staining with Congo red are strongly concordant methods of screening for amyloid in fat tissue⁸. Biopsy from other organs in those with subclinical amyloidosis was not done on ethical grounds.

Thus Indian patients with RA have a high prevalence of amyloid deposits, mostly subclinical. Early referral and early institution of therapy, with adequate control of disease activity, can probably reduce its prevalence. This cohort of patients may be examined after 5 to 10 years to determine the true significance, prognostic value, and therapeutic implications of subclinical amyloid deposits.

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Table 2. Amyloid detection in RA by ASFA. Comparison of 2 recent studies with the present study.

	Gomez-Casanovas, et al ⁷	Mansoury, et al ⁸	Present Study
No. of patients	313	112	113
Positive for amyloid, no. (%)	61 (19.4)	8 (7)	30 (26.5)
Subclinical amyloidosis, %	73	62.5	73.3
Fat deposit analysis, no	51	8	30
1+	12	7	23
2+	19	1	5
3+	20	0	2
Cutoff duration, yrs	> 5	> 5	> 5
Age, yrs, mean ± SD	61.4 ± 13.7	54 (41–65)*	46 ± 8.7
Male:female	3:58	0:8	8:22
Age at disease onset, yrs, mean ± SD	44.9 ± 14.4	32.5 (22–50)*	36 ± 10
Disease duration, yrs, mean ± SD (range)	16.8 ± 10.5 (5–49)	17 (12–25)*	10 ± 5 (5–22)
Followup, mo, mean ± SD (range)	69.7 ± 53.3 (0–175)	—	48 ± 36 (12–144)
RF positive, no. (%)	46 (75)	—	25 (83.3)
Erosive disease, no. (%)	57 (93)	—	25 (83.3)
CRP, mean ± SD, mg/dl	2.9 ± 2.9	9 (4–22)**	4.8 ± 4.0
No. of swollen joints, mean ± SD	5.5 ± 4.2	2 (0–7)*	5 ± 5
Arthroplasty, no. (%)	8 (13)	—	1 (3.3)
Renal insufficiency, no. (%)	9 (15)	0 (0)	0 (0)
Proteinuria, no. (%)	8 (13)	2 (25)	7 (23.3)
Receiving DMARD, no. (%)	57 (93)	6 (75)	25 (83.3)

ASFA test: Abdominal subcutaneous fat aspiration; RF: rheumatoid factor; CRP: C-reactive protein; DMARD: disease modifying antirheumatic drugs. *Median value (range). ** Normal value < 2.3 mg/dl.

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